

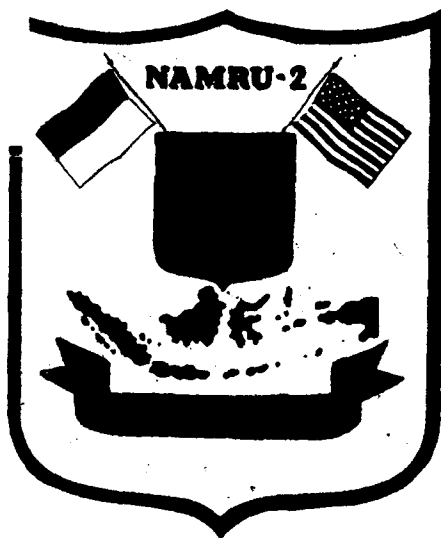
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DENGUE FEVER IN AMERICAN MILITARY PERSONNEL IN THE PHILIPPINES: CLINICAL OBSERVATIONS ON HOSPITALIZED PATIENTS DURING A 1984 EPIDEMIC

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INTRODUCTION

American military personnel frequently are stationed in areas out of the continental United States including some countries located in the tropics. During their tour of duty, which can last several years, they often are exposed to the common infectious diseases of these areas. In Southeast Asia, dengue (DEN) fever is one of the diseases encountered by this group. During the early part of this century, dengue fever was frequently reported among American troops in the Philippines with annual hospital admission rates of more than 100/1,000 in white enlisted men being common (Simmons *et al.*, 1931). Dengue also was reported among U.S. Army personnel in the Philippines during World War II (McCoy and Sabin, 1964).

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An outbreak of dengue was described among American troops stationed in Thailand in 1964 (Halstead *et al.*, 1969), and in a study of fevers of unknown origin among American soldiers in Vietnam, dengue was the most common diagnosis (Deller and Russell, 1967).

This study reports the clinical and virological data obtained in a group of American servicemen stationed at Clark Air Base in the Philippines who were hospitalized with dengue infections during a 1984 outbreak.

MATERIALS AND METHODS

Patient population and clinical studies.

All of the patients were active duty American military personnel stationed in the Republic of the Philippines at Clark Air Base (CAB) who were hospitalized at the CAB Regional Medical Center (RMC). Physical examinations were done by the medical personnel on duty, and signs and symptoms were recorded daily. Additional information on personal background and history of illness

was obtained by interviewing each patient. Routine blood chemistry tests were performed on most of the patients shortly after admission. Complete blood counts usually were done each day. Coagulation tests were performed on selected patients. All tests were performed in the hospital using standard clinical laboratory procedures.

Serology and virology.

Blood samples for serology and virus isolation were drawn by venipuncture on the day of hospital admission or as soon thereafter as practical. For some patients, additional blood samples for serology were taken during their hospital stay. Most of the patients had convalescent samples drawn between 2 to 4 weeks after the onset of illness, but in a few cases the convalescent samples were taken after a longer interval.

All sera were tested for antibody against all four DEN virus serotypes in a microtechnique hemagglutination-inhibition (HI) test (Clarke and Casals, 1958; Sever, 1962). A seroconversion was defined as a four-fold or greater change in HI antibody titer between the first and any subsequent sample against any of the serotypes, and was considered diagnostic. In patients who seroconverted and had a HI titer of $\geq 1:640$ the antibody response was classified as secondary (2°) indicating previous exposure to a flavivirus. If the HI titer in the convalescent sample taken 2 to 4 weeks after onset of illness did not exceed 1:320, the patient was classified as a primary (1°) responder. Patients who seroconverted but did not meet the criteria for either a 1° or 2° response were listed as unclassified. All serum samples from the same patient were tested simultaneously before final classification of the type of anti-

body response.

For virus isolation, two 25 cm² flasks containing confluent monolayers of *Aedes pseudoscutellaris* (LSTM-AP-61) cells were each inoculated with 0.02 ml of undiluted plasma from each patient's initial sample. The cells were incubated at 28 °C–30 °C in Mitsunashi-Maramorosch/Varma-Pudney (MM/VP12) medium that contained fetal calf serum and antibiotics (Varma *et al.*, 1974). Supernatant fluid from each flask in which the cells did not develop an obvious syncytial cytopathic effect (CPE) was passed to fresh LSTM-AP-61 cells after 1 week. Cells from these second passage flasks were harvested and fixed on teflon coated spot slides after 7 days. If the cells in any of the first and second passage flasks developed CPE at any time during the incubation, spot slides were prepared immediately.

The cells fixed on the spot slides were examined for DEN virus by a standard indirect fluorescent-antibody technique (IFAT). Monolayers which had not developed CPE were initially tested with a flavivirus group reactive monoclonal antibody (4G2). Cells that were positive in the initial IFAT and cells from all cultures with CPE were tested with type specific monoclonals for each serotype of DEN virus. The specific monoclonals used were 15F3 (DEN-1), 3H5 (DEN-2), 5D4 (DEN-3) and 1H10 (DEN-4) (Henchal *et al.*, 1982; Henchal *et al.*, 1983).

RESULTS

The 24 hospitalized patients in this study were all white males between the ages of 20–43 years ($\bar{x} = 29.1 \pm 6.7$). All patients

were admitted between 27 June and 31 August 1984, and reported having been ill from 1-8 days ($\bar{x} = 3.5 \pm 1.6$) prior to admission. The common symptoms that prompted the patients to seek medical attention were fever, headache, muscle and bone aches, weakness and loss of appetite. The number of days spent in the hospital ranged from 3-11 ($\bar{x} = 5.9 \pm 2.2$), and the total number of days that the patients reported being too ill to work, including time prior to hospitalization, during hospitalization and convalescent time after discharge, ranged from 3-18 ($\bar{x} = 8.7 \pm 3.8$).

The major signs and symptoms recorded while in the hospital are shown in Table 1. Fever, the most common sign, lasted an average of 6.1 ± 1.6 days from onset, and the mean maximum temperature was 102.0 ± 1.3 °F. A "saddle back" or diphasic fever pattern was not observed in any of the patients while in the hospital. The non-petechial rashes were described as being macular, maculopapular, acneiform or erythematous. The petechial rashes generally appeared late during the course of illness when the patient's temperature was normal or near normal. All patients also had a low platelet count on the day the petechial rash was noted (Table 2). In 6 of 9 cases this represented the nadir of their count.

In addition to petechial rashes, other hemorrhagic manifestations were recorded in a few cases. One patient each reported bleeding gums and passing a bloody stool, and 2 patients reported passing black stools prior to hospitalization. Another patient was initially admitted with a diagnosis of upper gastrointestinal (UGI) bleeding. An UGI aspirate contained coffee ground material as well as bright red blood, and blood also was detected by rectal examination. This patient later developed a petechial rash over the upper and

Table 1
Signs and symptoms recorded in 24 American Military Personnel hospitalized with confirmed dengue infections in the Philippines during June - August, 1984.

Finding	No. Cases (%)
Fever	24 (100.0)
Headache	23 (95.8)
Myalgias	22 (91.7)
Malaise	22 (91.7)
Anorexia	20 (83.3)
Bradycardia	19 (79.2)
Chills	19 (79.2)
Nausea	17 (70.8)
Dizziness	17 (70.8)
Nasal/Throat Congestion	17 (70.8)
Arthralgias	15 (62.5)
Hypotension	15 (62.5)
Rash (Non-Petechial)	13 (54.2)
Petechiae	9 (37.5)
Ocular Pain	9 (37.5)
Diarrhea	9 (37.5)
Vomiting	8 (33.3)
Abdominal Pain	8 (33.3)
Skin Flush	7 (29.2)
Itching or Desquamation	6 (25.0)
Adenopathy	4 (16.7)
Other*	1 or 2

* Includes ear pain, aberration of sense of taste and constipation.

lower extremities. The UGI bleeding could not definitely be attributed to the dengue infection, however, because he had a 3-4 year history of epigastric burning suggestive of peptic ulcer disease, and also had been taking aspirin regularly for several days prior to admission.

The occurrence of mild hypotension during the course of hospitalization was com-

Table 2

Appearance of Petechiae in relation to day of illness, temperature, and platelet count in Americans hospitalized with dengue during a 1984 outbreak at Clark Air Base in the Philippines.

Case	Day of Illness	Location of Petechiae ⁺	Max. Temp. (° F) of Day	Platelet Count (μl)
J.B.*	9	UE, LE, Abd, Back	99.6	69,000
T.N.	7	LE	100.4	73,000
K.S.	6	LE	102.6 [#]	88,000
D.C.	8	LE	99.0	22,000
R.P.	9	LE	99.0	52,000
M.H.*	6	LE	99.1	22,000
J.S.	6	UE, LE	99.6	72,000
R.S.	4	Soft Palate	102.6	55,000
J.N.	6	Chest	99.2	90,000

*Petechial rash may have been present prior to hospitalization. The data presented is from the first hospital day.

⁺UE = upper extremities; LE = lower extremities and Abd = Abdomen

[#] Temperature returned to normal later on the same day.

mon. A systolic reading of ≤ 90 mmHg was recorded in 15 patients, and four of these had readings of ≤ 80 mmHg. Five of the patients with hypotension also had a narrowing of pulse pressure to ≤ 20 mmHg. The only patient with signs of peripheral circulatory failure was the one with UGI bleeding. Clinically, he was afebrile and lethargic with cold clammy skin on initial examination in the emergency room. His admission blood pressure of 60/40 increased to 100/70 with intravenous fluid challenge and remained stable for the duration of his illness.

Leucopenia and thrombocytopenia were the most common hematological abnormalities (Table 3). Of the 23 patients with leucopenia, all but two had counts below $4.5 \times 10^3/\mu\text{l}$ on the day of hospital admission. The leucopenia persisted for ≥ 3 days in 18 patients and ≥ 5 days in 7 of these

patients. A relative lymphocytosis ($\geq 50\%$ lymphocytes) was recorded in 20 patients, and atypical lymphocytes were observed in all of these cases (\bar{X} max = $13.4 \pm 9.4\%$). All of the patients presented with a low platelet count sometime during hospitalization. A platelet count of $\leq 150,000/\mu\text{l}$ was recorded in 10/22 patients on the day of admission and five of these patients had counts of $\leq 100,000/\mu\text{l}$. The duration of thrombocytopenia ($\leq 150,000/\mu\text{l}$) was ≥ 3 days in 17 patients and ≥ 5 days in 6 of these cases.

The highest hematocrit (Hct) reading for each patient while in the hospital ranged from 41–53% (\bar{X} max = 46.4 ± 2.6). Only 13 of the patients had baseline or recovery Hct readings to compare with their hospital readings. The greatest degree of hemoconcentration recorded was 17.8% in a patient whose highest hospital Hct was 46.0% with a Hct taken 11 days after hospital discharge

of 39.2%. The highest hospital Hct corresponded to the nadir of his platelet count (22,000/ μ l).

Other hematologic abnormalities included a prolonged partial thromboplastin time (PTT) in 6/11 of the patients tested, and a mildly elevated level of fibrin split products in 4/6 of the patients tested. The prothrombin time was within normal limits for the same 11 patients that were tested for PTT. The most frequent abnormal blood chemistry findings were hyponatremia in 10/20, a low total protein in 7/18 and elevated levels of aspartate aminotransferase in 7/18 of the patients tested.

The diagnosis of dengue was confirmed serologically and by virus isolation in 12/24 of the patients. Of the remaining 12 cases, 10 were confirmed by serology only, and 2 by virus isolation only. Of the 22 patients confirmed serologically, 16 (72.7%) showed a 2° type of antibody response in the HI test. Three had a 1° type of response, and

the antibody response could not be classified in 3 of the cases that seroconverted. Seven of the patients showing a 2° type of antibody response and one patient with an unclassified antibody response reported having been vaccinated previously for yellow fever (YF). Three patients who reported never being vaccinated for YF all had a 1° antibody response. The YF vaccine status of the remaining patients was unknown. Seven isolates of DEN 1, 4 isolates of DEN 3 and 3 isolates of DEN 2 were recovered from the patients.

DISCUSSION

All of our patients were clinically classified as classical DF. None met the World Health Organization (WHO) criteria for dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), the more severe and sometimes fatal forms of dengue disease seen in children in Southeast Asia and particularly associated with 2° dengue infections (WHO, 1986). The 2° type of antibody response seen in 16 of our patients could have been the result of sequential dengue infections in some instances since the average length of residence in the Philippines for these patients was 19 months. A more likely reason, however, was prior vaccination for YF. Infection with dengue virus after YF vaccination has been documented to cause a 2° type of antibody response in the HI test (Bancroft *et al.*, 1984). Although the numbers for comparison are small, the patients with a 2° type of antibody response did not appear to differ clinically in any way from the other patients. Likewise, no clinical differences were apparent among the patients that were infected with any of the three serotypes of dengue virus found in the study patients.

Table 3

Lowest white blood cell and platelet counts recorded on 24 American Military Personnel hospitalized with dengue during 1984 at Clark Air Base in the Philippines.

Cell Count (X10 ³ / μ l)	No. Cases
WBC:	
<2.5	14
2.5 - 3.4	8
3.5 - 4.4	1
≥4.5	1
Platelets:	
<50	7
50 - 100	13
101 - 150	4

Most of the signs and symptoms we recorded in this study have been reported during other dengue outbreaks in similar population groups in this region. The difference in the frequency of occurrence and intensity of some manifestations, however, is noteworthy. A "saddleback" or diphasic fever curve was not recorded for any of our patients. This is consistent with the observations on American soldiers in Vietnam who were hospitalized with dengue (Deller and Russell, 1967). In contrast, a diphasic fever curve was common among American military volunteers experimentally infected with either DEN 1 or DEN 4 in early clinical studies conducted in the Philippines (Simmons *et al.*, 1931; Siler *et al.*, 1926). The fact that patients were closely monitored from the very onset of fever in these latter studies may account for the frequent observation of a diphasic fever curve. In one of the volunteer studies, the investigators noted that in "natural" dengue cases observed in the hospital during the same period of time that the experimental infections were being conducted, the diphasic type of fever curve did not occur (Siler *et al.*, 1926).

Petechial rashes occurred in over a third of our patients. In the early studies with American troops in the Philippines, only 1 out of 129 volunteers was noted to develop a petechial eruption (Simmons *et al.*, 1931; Siler *et al.*, 1926). In the Vietnam study, 13% of the American soldiers hospitalized with dengue had petechial rashes (Deller and Russell, 1967), but in a study of 25 American troops with confirmed dengue infections in Ubol, Thailand, no petechiae were reported (Halstead *et al.*, 1969). In this same Thailand report, purpura was observed on the lower extremities in 3/68 American and European adults infected with dengue in Bangkok. In another study from Bangkok,

3/5 hospitalized white Americans were reported to have petechiae (Nelson and Bierman, 1964), but whether or not these 5 patients were representative of an apparently larger group of Americans admitted to the same hospital with dengue during the same period of time is not clear (Halstead *et al.*, 1969; Nelson and Bierman, 1964).

The high frequency of leucopenia seen in our patients has been a common finding in other studies on similar groups of patients; however, the strong "shift to the left" so clearly documented in the DEN 1 studies with American military volunteers was not observed in our patients (Simmons *et al.*, 1931). A high frequency of thrombocytopenia, particularly a count $\leq 100,000/\mu\text{l}$ has not been commonly reported in non-resident Caucasians with dengue in Southeast Asia. This may partially be because the opportunity or facilities to routinely monitor platelet counts were not available. In the experimental DEN 1 infection studies done in the Philippines, daily platelet counts were done on only two American military volunteers and no substantial reductions were noted (Simmons *et al.*, 1931). In a study in Bangkok, platelet counts between 60,000 - 90,000/ μl were reported in 5 Americans hospitalized with dengue between 1962 - 1963, but how commonly a count this low occurred among the total population of Americans hospitalized during this outbreak is not specified (Nelson and Bierman, 1964). Another study done in Bangkok during the same period reported platelet counts between 50,000 - 100,000/ μl for three U.S. Peace Corps Volunteers who formed part of a group of 68 American and European adults with confirmed dengue infections (Halstead *et al.*, 1969).

As far as we are aware, the frequent occurrence of mild hypotension in adults with

classical dengue fever has not been reported from Southeast Asia. The frequency of mild hypotension in our patients suggests that it should be considered as part of the normal spectrum of clinical signs associated with this illness.

The severity of the disease seen in these 24 hospitalized cases may have represented only the extreme end of the clinical spectrum of a larger number of dengue infections that occurred in the community at large during this outbreak. Another 10 cases of milder but typical dengue-like disease were detected among military personnel treated as outpatients at the RMC during this period (Hayes, unpublished); however, we were not able to conduct a population based survey to determine the number of unreported dengue infections among the approximately 9,000 military personnel stationed at CAB.

We did conduct a very limited survey during November and December, 1984, on healthy troops reporting to the RMC for routine physical examinations (Hayes, unpublished). Among 113 individuals, 7 (6.2%) had detectable HI antibody against dengue virus. None of them had a recent history of a dengue-like illness; but it is possible that they had experienced a recent subclinical dengue infection or an infection resulting in atypical dengue disease. The frequency of any recent febrile illness was no greater, however, in the individuals with HI antibody than in the group without antibody. A previous study on American and Australian troops stationed in Thailand indicated that the ratio of overt disease to total infection was 1:1 after infection with DEN 1 (Halstead *et al.*, 1969), the predominate serotype isolated from our patients. Based on these data, we may have detected most of the dengue infections that occurred in this population, particularly since military per-

sonnel have access to free medical care, and must report for medical evaluation to be excused from duty because of illness.

Comparing our clinical data with other reports on dengue infections in non-immune adult Caucasians residing in Asia suggests that the clinical expression of disease may vary in different outbreaks. This study clearly documents that thrombocytopenia, mild hypotension and petechiae can be prominent manifestations of classical dengue fever in this type of patient. Although not life threatening, the illness seen in these 24 patients was highly incapacitating as shown by an average period of hospitalization of 6 days.

SUMMARY

From June - August, 1984, 24 American military personnel were hospitalized with dengue (DEN) at Clark Air Base in the Philippines. Their infections were confirmed by serology using the hemagglutination-inhibition test and/or by virus isolation in *Aedes pseudoscutellaris* cell cultures. Most of the patients had a secondary type of antibody response probably reflecting prior vaccination against yellow fever. Three serotypes of DEN virus were isolated; 7 isolates of DEN 1, 4 isolates of DEN 3 and 3 isolates of DEN 2.

All of the patients were Caucasian males between the ages of 20-43 years. All of the cases were clinically diagnosed as classical dengue fever. A platelet count of $\leq 100,000/\mu\text{l}$ was a common finding (83.3%); however, hemoconcentration was not documented. Other major findings were the occurrence of mild hypotension (62.5%) and petechiae (37.5%). One patient presented with shock and upper gastrointestinal bleeding, but his diagnosis was complicated by a

history of epigastric pain and use of aspirin. Although all of the patients fully recovered, the severity of illness was clearly documented by the average-length of hospitalization (5.9 days) and average time absent from work (8.7 days).

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